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Specification and Drawing, as originally filed, with Application for Patent Serial No: **2,243,669**, on July 21, 1998, by **BIOS AGRICULTURE INC. AND MCGILL UNIVERSITY**, assignee of Donald L. Smith, Pan Bo, Yinghai Deng, Pierre Migner and Feng Zhang, for "Composition For Accelerating Plant Seed Germination, Plant Growth And Methods Using Same".

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Date

ABSTRACT OF THE DISCLOSURE

The present invention relates to agriculture. More specifically, the invention relates to plant seed germination and plant growth. Even more specifically, the present invention relates to compositions which accelerate plant seed germination and plant growth of numerous types of plants and to methods using same.

TITLE OF THE INVENTION

COMPOSITION FOR ACCELERATING PLANT SEED GERMINATION, PLANT GROWTH AND METHODS USING SAME.

FIELD OF THE INVENTION

The present invention relates to agriculture. More specifically, the invention relates to plant seed germination and plant growth. Even more specifically, the present invention relates to compositions which accelerate plant seed germination and plant growth of numerous types of plants and to methods using same.

BACKGROUND OF THE INVENTION

SoyaSignal® is a product, designed by Bios Agriculture Inc., to initiate soybean nodulation and nitrogen fixation under cool soil temperatures. SoyaSignal has been tested in the last 4 years and over twenty different locations in both Canada and USA. As the SoyaSignal technology increased, the final soybean grain yield by 11.1%, farmers in both countries have accepted this product.

Initial recognition between *B. japonicum* and soybean involves exchange of molecular signals (Stacey et al, 1995). Legume roots secrete phenolic compounds (Dakora & Philips, 1996; Peters & Verma, 1990), largely from the area of root hair emergence, which act as chemo-attractants to (brady)rhizobia (Nap & Bisseling, 1990), and activate the *nod* genes. Flavones, isoflavones, and flavanones have been identified as the inducing molecules for (brady)rhizobial chemotaxis and for expression of *nod* genes, e.g. genistein, daidzein and several related compounds in soybean (Peters & Verma, 1990). These plant-to-

bacteria signal compounds cause expression of the bacterial *nod* (also *nol* and *noe* genes) very rapidly (only a few minutes after exposure) and at very low concentrations (10^{-7} to 10^{-8} M) (Peters et al., 1986). Generally this is through an interaction with *nodD*, which activates the common *nod* genes, although the situation may be more complex, as is the case in *B. japonicum*, where $nodD_1$, $nodD_2$ and *nodVW* are involved (Gillette & Elkan 1996; Stacey 1995).

Among the products of the *nod* genes (Spaink, 1995; Stacey, 1995) induced by the plant phenolic signal molecules are various enzymes involved in the synthesis of a series of lipo-chitooligosaccharides (LCOs). These LCOs act as bacterium-to-plant signals, inducing expression of many of the early nodulin genes (Long, 1989). This results in root hair deformation (including curling) cortical cell division leading to initiation of nodule meristems, secretion of additional *nod* gene inducers, and initiation of infection threads (Verma, 1992). These bacterium-to-plant signals exert a powerful influence over the plant genome and, when added in the absence of the bacteria, can induce the formation of root nodules (Truchet et al., 1991). Thus, the bacteria-to-plant signals can, without the bacteria, induce all the gene activity for nodule organogenesis (Denarie et al., 1996; Heidstra & Bisseling, 1996).

The relationship between environmental variables, such as low RZT and pH, and the interplay of molecular signals has only recently become a subject of investigation. For example, soybean plant has less synthesis abilities of isoflavones under cool soil temperature, whereas the higher isoflavone concentration is needed to turn on the *nod* genes of *B. japonicum* (Zhang and Smith 1995 and 1997). To overcome the negative effect of low temperature on the early event of symbiotic

nitrogen fixation, the SoyaSignal technology has been designed (Canadian application number 2,179,879). The major components of SoyaSignal are isoflavones and LCOs.

While the effect of isoflavones and LCOs on nodulation, nitrogen fixation, growth and protein yield of legumes, such as soybean, has been shown to compensate for stress conditions of the environment, their effect on growth of non-legumes is unknown. In fact, the role of such legume-specific-related molecules on non-legumes has never been assessed on non-legume plants.

There thus remains a need to assess the effect of isoflavones and/or LCOs on plant germination and/or growth of plants and especially of non-legume plants.

The present invention seeks to meet these and other needs.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

SUMMARY OF THE INVENTION

The invention concerns a composition for enhancing seed germination of plants and growth thereof. More specifically, the present invention relates to a composition of SoyaSignal which can increase soybean seed germination and plant growth in addition to acting as a trigger to initiate soybean symbiotic nitrogen fixation. Surprisingly, the compositions of the present invention act not only on legumes such as soybeans but on plants in general as exemplified with corn and cotton. In a particular set of experiments, a composition of the present invention

comprising isoflavones and LCOs was shown to significantly enhance early plant growth.

Broadly, the present invention further relates to compositions comprising isoflavones and LCOs for enhancing seed germination of plants and growth thereof.

The invention in addition relates to methods for enhancing the germination and/or growth of plants comprising an incubation of a seed of a plant with an effective amount of isoflavones and LCOs for a sufficient time and under conditions which enable an increased germination of the seed and/or an increased growth of the plant.

The applicant was the first to show that isoflavones and LCOs had an effect on non-legume germination and plant growth.

While the germination and/or plant growth enhancing capabilities of the composition of the instant invention are demonstrated with corn and cotton, they are applicable to other plants such as, for example, potato tubers.

Similarly, while the present invention has been demonstrated with low RZT, the composition and methods of the present invention should find use in stimulating germination and/or growth under environmental field conditions. Non-limiting examples thereof include water stress, pH and high temperature.

It shall also be understood that compositions other than SoyaSignal, comprising isoflavones (and/or flavones and/or flavanones) and LCOs, are also encompassed within the scope of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1 shows the germination enhancing effect of a composition according to the present invention.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Surprisingly, we have shown that the addition of SoyaSignal, in the presence of isoflavones and LCOs accelerates the germination of corn seeds, whereas isoflavone solutions alone do not. Presumably this effect was due to the LCOs produced by the presence of isoflavones and *B. japonicum* cells. When the seedlings were harvested (still at the mesocotyl stage) they were 44% longer and 33% as heavier, than the plants which had not received the genistein-*B. japonicum* treatment (Figure 1). In addition, we have shown that the rate of cottonseed germination is also accelerated by the application of SoyaSignal. The germination rate of cotton seeds treated with SoyaSignal at the rate of 0.66 ml/kg seed increased by 145% compared to those control seeds that only treated with pure water. Both the corn and cotton work were conducted at low temperatures, 15°C for corn seeds and 17.5°C for cottonseeds.

From our field trial this year, the time of tasselling of sweet corn treated with SoyaSignal (planted on May 6 on the Experimental Farm of McGill University, Quebec) was 1 to 2 days earlier compared to untreated plants. Soybean seeds that received SoyaSignal (planted on June 22 in Martinsville, Illinois) emerged 8 hour earlier compared to control seeds while the first trifoliar fully expanded 1 day earlier. At the agronomy farm of Purdue University, IN. soybean plants planted in early June and observed in early July had already grown to one stage further in their development (V6) compared to the control plants (V5). In a farmer trial (in Jackson, Illinois), plants that received SoyaSignal had much more nodule on the secondary roots and were 10% taller than untreated plants.

It is still not fully understood why SoyaSignal works on plant in this way. Our general understanding of the role of LCOs in signalling during the establishment of the legume-rhizobia symbiosis was described above. When added to the appropriate legume, LCOs can cause the induction of nodule meristems. Two previous publications have shown that LCOs can induce cell cycle activities in other systems. De Jong et al. (1993) showed improved embryogenesis in a carrot embryogenesis system. Röhrig et al. (1995) showed increased cell division in a suspension of tobacco protoplasts at concentrations as low as 10^{15}M. The possible explanation of our observation may be as the following. First, LCOs might be normal signal molecules in higher plants, so that exogenously supplying them simply increases their levels and, therefore, the activity of the things they would normally regulate. Second, there may be an endogenous class of signal molecules which have play important roles in plant development, and have a conformation similar to

those of LCOs. Without being limited to a particular theory, the present invention is the first to have identified a germination and/or plant growth promoting effect of isoflavones and LCOs on non-legume plants.

The crops, such as soybean, corn and cotton evolved in relatively warm climates and, as a result, have high base temperatures for germination, being 10°C for both soybean and corn and 14°C for cotton, respectively. These high base germination temperatures lead to slow emergence after planting, resulting in slow leaf ground cover early in the season, which in turn leads to poorer early season light interception, poorer competition with weeds (and therefore greater need for herbicide application) and increased soil erosion during heavy rainfall events. Using SoyaSignal as a plant growth regulator can partially overcome the negative effects of environmental stress conditions, such as low soil temperature on crop seed germination and plant development. These will improve the production of tropical and subtropical origin crops in temperate zone and may extend their production into other short season areas.

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

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WHAT IS CLAIMED IS:

1. An agricultural composition comprising isoflavones and LCOs for enhancing plant seed germination and/or plant growth of a plant together with an agriculturally suitable carrier.

2. The composition of claim 1, wherein said plant is a non-legume.

3. The composition of claim 2, wherein said plant is selected from the groups selected from graminae, solonacae and malvacae.

4. The composition of claim 3, wherein said plant is selected from the group comprising corn, cotton, tomato, potato and tobacco.

5. A method to enhance plant seed germination and/or plant growth of a plant comprising an incubation of said seed and/or plant with an agriculturally effective amount of the agricultural composition of claim 1.

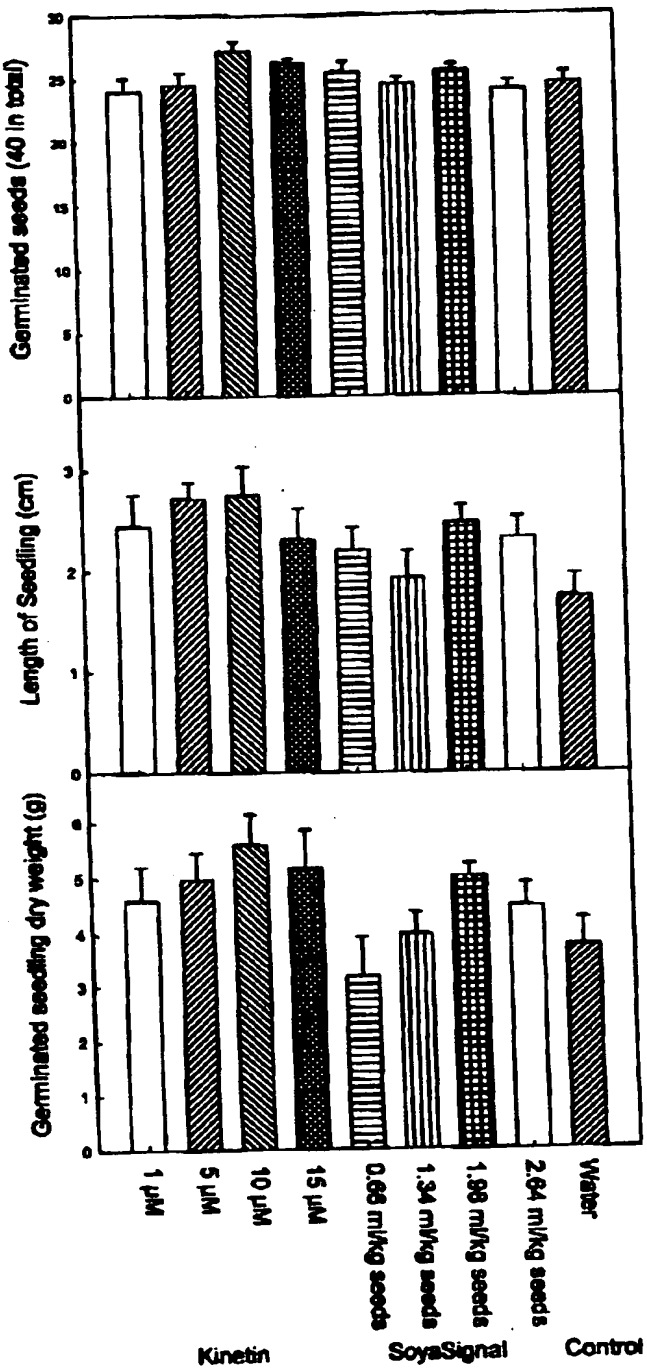
Germinated seeds (40 in total)
Length of Seedling (cm)
Germinated seedling dry weight (g)
1 µM
5 µM
10 µM
15 µM
0.66 ml/kg seeds
1.34 ml/kg seeds
1.98 ml/kg seeds
2.64 ml/kg seeds
Water
Kinetin
SoyaSignal
Control

FIGURE 1